# INTRODUCTION

## PAIN

Tissue damage caused by injury, disease, or inflammation releases endogenous chemicals, called algogenic, algesic, or pain-producing substances, into the extracellular fluid that surrounds the nociceptors. These substances include $H^+$, $K^+$, serotonin (5-HT), histamine, prostaglandins (PGs), bradykinin, substance P (sP), and many others. They play a causal role in pain associated with inflammation, trauma, bone tumors, ischemia, and a variety of other pathophysiologic conditions. In addition to direct excitatory action on the membrane of nociceptors, these agents may have an indirect excitatory action by altering the local microcirculation. The algesic substance can cause increase capillary permeability and either vasoconstriction or vasodilation (Raj, 1996).

### Location of algesic substances

5-HT, histamine, $H^+$, $K^+$, PGs, and other members of the arachidonic acid cascade are located in tissues; kinins are in plasma; and sP is in nerve terminals. Histamine is found in platelets, basophils, and the granules of mast cells; 5-HT is present in mast cells and platelets. Release of these amines may

be induced by mechanical injury, noxious heat, radiation, and certain byproducts of tissue damage, most notably neutrophil lysosomal materials, thrombin collagen, and epinephrine. Tissue damage also induces release of lipidic acids of the arachidonic acid cascade, such as PGs and leukotrienes (LTs) including slow-reacting substance of anaphylaxis (SRS-A) (Raj, 1996).

**Bradykinin**

Bradykinin is a byproduct of the cascade that is triggered by the activation of factor XII of the Hageman clotting system by exposure to negatively charged surfaces such as collagen. This activation results in the conversion of the enzyme prekallikrine to kallikrein, which then acts on the bradykinin precursor kininogen, resulting in the release of bradykinin into the tissue. Bradykinin produces increased vascular permeability, promotes vasodilation, produces leukocyte chemotaxis, and activates nociceptors. The action of bradykinin on nociceptors is potentiated by PGs present in the injured tissue compartment (Raj, 1996).

**Substance P (sP)**

Substance P (sP), which is considered to be a neurotransmitter in pain fibers, is released at the site of injury. Although sP is not itself an algesic, it increases the permeability of blood vessels. This produces a leakage of fluid into the surrounding tissues, which provides for wider diffusion of the algesics. In this manner a larger area becomes painful (Insel, 1991).

**Prostanoids**

The PGs are biosynthesized in the body from certain polyunsaturated essential fatty acids, among which is the abundant arachidonic acid. Arachidonic acid is the precursor of PGs and thromboxanes (TXs), which are qualitatively and quantitatively important prostanoids. Precursor fatty acid normally does not occur free in the cell; it is esterized to phospholipids. Conversion into PGs and release of the related substances thus start with the liberation of the fatty acid by the action of a phospholipase $A_2$ which releases the cell-membrane-derived arachidonic acid. A number of stimuli are known to lead to the activation of phospholipase and thus to increased PGs synthesis. These stimuli include norepinephrine and dopamine, which stimulate synthesis of the cellular

phospholipids, in part by releasing the nonesterified free fatty acid precursors. Cyclooxygenase, which is membrane-bound enzyme, acts as substrate to synthesize PGs. Significantly, in damaged skin there is a marked elevation of prostanoid level. Agents such as aspirin and indomethacin inhibit cyclooxygenase and thus cause depletion of these lipidic acids, resulting in relief of pain (Raj, 1996).

The capacity of PGs to sensitize pain receptors to mechanical and chemical stimulation has been confirmed by electrophysiological measurements and appears to result from a lowering of the threshold of the polymodal nociceptors of C fibers (Perl, 1976). In general, the aspirin-like drugs or nonsteroidal anti-inflammatory drugs (NSAIDs) do not affect the hyperalgesia or the pain caused by direct action of PGs, consistent with the notion that it is their synthesis that is inhibited (Insel, 1991).

**Sensitization**

Sensory end organs are assumed to have certain thresholds that remain constant despite change in conditions and states with repeated stimulation, however, most sensory organs (including low-threshold C fibers) become fatigued and less responsive. High-threshold polymodal C fibers involved in

nociception show the opposite response. With repeated stimulation, these nerve endings displayed enhanced sensitivity, lowered threshold to stimulation, and prolonged and enhanced response to the stimulation (after discharge). This phenomenon is called sensitization. Tissue damage caused by injury or disease produces a similar type of sensitization at the site of injury, called primary hyperalgesia, that is also characterized by a lowered pain threshold, increased sensitivity to suprathreshold stimuli, and spontaneous pain.

After the injury, a much larger area of hyperalgesia and allodynia that surrounds the site of injury develops, called secondary hyperalgesia. The mechanisms of primary hyperalgesia and secondary hyperalgesia after injury or inflammation are probably similar and involve endogenous biochemical agents (Raj, 1996).

**Transduction of painful stimuli**

Although the precise mechanism by which endogenous chemicals participate in peripheral transduction of nociceptive stimuli into nociceptive impulses is not known, these substances may initiate three mechanisms: (1) those that activate nociceptive afferent fibers and produce pain by local application (e.g., bradykinin, acetylcholine, and potassium); (2)

those that facilitate the pain evoked by chemicals and physical stimuli by sensitization of nociceptors but are ineffective in evoking pain themelves (e.g. PGs); and (3) those that produce local extravasation (e.g. sP). In addition to these mediators from extraneural sources, sP and other peptides may have a role in influencing the milieu of the peripheral afferent terminal and thus, indirectly, in the transduction of nociceptive information (Raj, 1996).

**Drugs used to treat pain**

Several groups of compounds are used to relieve pain, depending on the severity and duration and on the nature of the painful stimulus. These drugs are classified in several ways. Drugs used to relieve pain without causing unconsciousness are called analgesics and are subdivided into two groups according to their ability to relieve mild, moderate, or severe pain. Mild analgesics are termed nonnarcotic and include such agents as aspirin and NSAIDs. The narcotic (opioid) analgesics used to control moderate to severe pain, of which morphine is the prototype (Dewey, 1994). Aspirin and NSAIDs are thought to act primarily in the periphery. In contrast to morphine-like drugs, which act at specific receptor

sites in the central nervous system (CNS) to block pain transmission (Fields, 1987).

**INFLAMMATION**

The inflammatory process involves a series of events that can be elicited by numerous stimuli (e.g., infectious agents, ischemia, antigen-antibody interactions, and thermal or other physical injury). At the microscopic level, the response is usually accompanied by the familiar clinical signs of erythema, edema, hyperalgesia and pain. Inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms: (1) an acute transient phase, characterized by local vasodilatation and increased capillary permeability; (2) a delayed, subacute phase, most prominently characterized by infiltration of leukocytes and phagocytic cells; and (3) a chronic proliferative phase, in which tissue degeneration and fibrosis occur (Insel, 1991).

Many mediators of the inflammatory process have been identified, such as histamine, kinins, PGs, interleukotriene-1 (IL-1), eicosanoids, etc.

The important role of histamine in inflammation, mainly in acute phase, such as increase in blood flow and content, increase in vascular permeability, and edema formation has

long been accepted (Owen, 1987). Histamine can also cause pain and itching (Douglas, 1985). An alternative role of histamine might be a co-mediator of inflammation, for example, histamine and prostaglandin-$E_2$ ($PGE_2$) possess synergistic effect on either increase of microvascular permeability or pain production. Histamine may be chemotactic specifically for eosinophil (Clark, *et al.*, 1975).

Kinins cause vasodilation, increase capillary permeability and provoke pain as well as modulate mobilization of leukocytes. Kinins appear to participate to the acute and chronic phases of the inflammatory reactions and are considered as blood-borne pro-inflammatory agents since they increase the synthesis and release of PGs (Regoli, 1987).

In the inflammatory reaction platelet activating factor (PAF) has a significant role both directly by acting on the cardiovascular system causing e.g. vasodilation and increased vascular permeability, and indirectly by stimulating inflammatory cells (Page, *et al.*, 1984).

Interleukin-1 (IL-1) is a strong pro-inflammatory agent in human skin causing swelling, erythema, and local hyperthermia, which is accompanied by considerable accumulation of neutrophils. It can also act as a chemotactic substance for many cell types such as neutrophils, monocytes, T and B-

lymphocytes. IL-1 is considered as an endogenous pyrogen, which mediates pyrexia through the production of $PGE_2$ (William and Higgs, 1988).

Eicosanoids are used to describe a range of widely different chemical substances including prostanoids and LTs, which are oxidative metabolites of arachidonic acid. There are two principal enzymatic pathways of arachidonic acid oxygenation involved in the inflammatory process i.e. the cyclooxygenase pathway that produces PGs and the 5-lipoxygenase that produces LTs (Fig. 1). $PGE_2$ and prostacyclin ($PGI_2$) are the most important inflammatory mediators among other cyclooxygenase products. They are potent vasodilator and hyperalgesic agents (Salmon and Higgs, 1987). They are present in inflamed tissues in sufficient concentrations to account for the erythema and increased sensitivity which is characteristic of acute inflammation (Solomon, *et al.*, 1968). They can potentiate the increase in vascular permeability caused by histamine and bradykinin. Aspirin can inhibit $PGE_2$ synthesis *in vivo* and can then reduce edema (Higgs, *et al.*, 1984) and this specific action can explain the analgesic, antipyretic and anti-inflammatory effects of aspirin and other NSAIDs (Salmon and Higgs, 1987). Thromboxane $A_2$ ($TXA_2$) is one of the cyclooxygenase products which mediates platelet

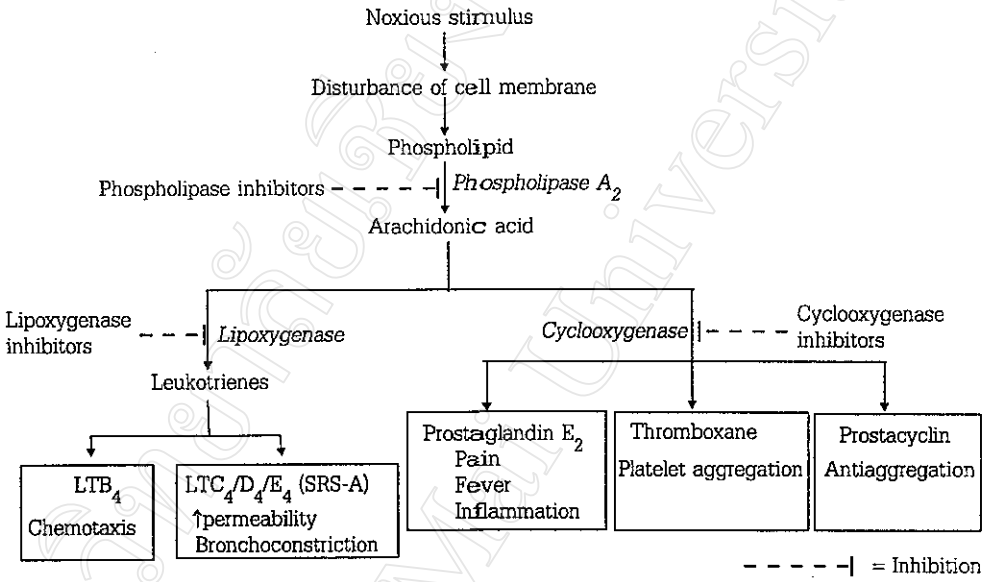


**Fig. 1** Scheme of the major metabolic transformation of arachidonic acid

aggregation and cause platelet release of many physiological active substances such as 5-HT (Sheldon, 1984).

LTs are a novel group of biologically active mediators derived from arachidonic acid via lipoxygenase enzyme. $LTB_4$ is a potent chemotactic agent for polymorphonuclear leukocytes (PMNs). $LTC_4$, $LTD_4$ and $LTE_4$ (SRS-A) are potent bronchoconstrictors, which can produce erythema and whealing in human skin by increasing vascular permeability (Camp and Greaves, 1987).

**FEVER**

Fever is a result of infection or one of the sequels of tissue damage, inflammation, graft rejection, malignancy, or other disease states. A common feature of these conditions is the enhanced formation of cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), interferons alpha and beta (INF-α and INF-β) or tumor necrosis factor alpha (TNF-α). $PGE_2$ acts within the hypothalamus to produce the resultant elevation of body temperature by a process that appears to be mediated by cyclic AMP. The action of aspirin-like drugs or NSAIDs in promoting a return to the normal set-point for temperature could be explained as being due to their inhibition of prostaglandin

synthesis (Dascombe, 1985). Once there has been a return to the normal set point, the temperature-regulating mechanisms, dilatation of superficial blood vessels, sweating, etc. then operate to reduce temperature. The evidence for this scenario includes the ability of $PGE_2$ to produce fever when infused into the cerebral ventricles or when injected into the hypothalamus. In addition, fever is a frequent side effect of prostaglandins when they are administered to women as abortifacients. The fever caused by agents that enhance the synthesis of IL-1 and other cytokines, but not that caused by PGs, is reduced by aspirin-like drugs. Normal body temperature is not affected by aspirin and NSAIDs Milton (1982).

**ANIMAL MODELS USED IN THE PRESENT STUDY**

The writhing response induced in mice by an intraperitoneal injection of a noxious agent is commonly used as a basis for testing analgesic activity. This response consists of a contraction and elongation of abdominal wall, accompanied by twisting of the trunk and followed by extension of the hind limbs (Collier, *et al.*, 1968). The latency and duration of writhing response depends on the characteristic of the challenge substance. Acetic acid is supposed to act indirectly, possibly by liberating endogenous substances that excite pain

nerve ending. Inhibition of response by analgesic drugs such as aspirin or NSAIDs in this test was found to be well correlated with clinical results in human (Taber, *et al.*, 1969).

Tail-flick test, a method determining the central acting analgesic, was demonstrated by D'Amour and Smith (1941). Tail-flick response appears to be a spinal reflex that is modulated by a supraspinal inhibitory mechanism (Harris, *et al.*, 1969). Its advantage lies in its selectivity as all the potent narcotic or morphine-like analgesics show inhibitory activity in this model (Howes, *et al.*, 1969).

The formalin test in mice is sensitive to NSAIDs anti-inflammatory drugs and other mild analgesics. The test possesses two distinctive phases, possibly reflecting different types of pain. The earlier phase reflects direct effect of formalin on nociceptors (non-inflammatory pain), whereas the late phase reflects inflammation (Hunskaar and Hole, 1987). By contrast with acetic acid-induced writhing, this model is a highly sensitive but not a very selective pain test, with false positives occurring with sedative, muscle relaxant and other pharmacological activities (Elisabetsky, *et al.*, 1995).

Ear edema induced in rats by arachidonic acid (AA) or ethyl phenylpropiolate (EPP) was suggested to serve as a more useful model for the testing of anti-inflammatory activity

(Young, *et al.*, 1984). This experiment seems to be a useful model for the rapid *in vivo* screening of agents since only a small amount of a test substance is needed. By using both edema inducers (AA and EPP), the mechanism involved can be suggested. Leukotriene are involved in the formation of edema when AA is used as inducer whereas kinins, 5-HT and PGs are released in EPP-induced ear edema (Brattsand, *et al.*, 1982 and Young, *et al.*, 1984). This assay has been accepted to serve as a preliminary screening test for anti-inflammatory activity because the response is easily measured (Chang, *et al.*, 1986).

Experimental models of fever have used microbial and antigenic agents that provoke inflammation and all have implicated leukocytes, important participants in the inflammatory response, as responsible for endogenous pyrogen release (Atkins and Bodel, 1974). Yeasts either directly or by releasing pyrogenic material, activate cells within the body to synthesize and release an endogenous pyrogen. The cells capable of producing endogenous pyrogen include neutrophils, monocytes and lymphocytes (Milton, 1982). It is propable that these cells play an important role in fever associated with disease where chronic inflammation is a prominent feature (Atkins and Bodel, 1974). Milton (1982) found that $PGE_2$ is an endogenous modulator responsible for fever and that antipyretic

drugs such as aspirin and paracetamol produce their action by inhibiting PGs biosynthesis and release.

## LITERATURE REVIEW OF *DIOSPYROS VARIEGATA* KRUZ.

*Diospyros variegata* Kruz. belongs to the family Ebenaceae. Its Thai name is Phayaa-raak-dam. The tree is up to 20 m high. The leaf is simple, alternate, dark green, oval-shaped, 5-11 cm wide and 15-30 cm long. The flowers are yellow and solitary. The berry fruit is green and turn black after ripening. The leaves and fruit are illustrated in Fig. 2

In Thai folklore medicine, stems of *D. variegata* are boiled in water and the water extract is heated until dark thick mass is formed, which is cooled down and sliced into pieces, a decoction of these slices is used as a tea for the management of pain and inflammatory conditions (Panthong, *et al.*, 1986).

Pharmacological study of the extract from the root of *D. variegata* revealed that the extract did not possess antihistaminic and antispasmodic activity when tested using isolated ileum of guinea-pig and had no hypotensive action in anesthetised dogs (Mokkhasamit, *et al.*, 1971).

Phytochemical study of the root of *D. variegata*, showed that the plant did not contain any alkaloid substance (Congdon, *et al.*, 1981).

**Fig. 2** Leaves and fruit of *Diospyros variegata* Kruz.

Mokkhasamit *et al.* (1971) observed acute toxicity of the crude methanol extract of the root of *D. variegata* in mice and found no toxicity after an oral and intraperitoneal administration of different doses i.e. 1, 3 and 10 g/kg. The dose of 10 g/kg in mice is about 200,000 times the dose used in humans in Thai traditional practice (0.05 mg/kg).

**PURPOSE OF THE STUDY**

The purpose of this study was to evaluate the analgesic, antipyretic and anti-inflammatory activities of the extracts from the stem of *D. variegata* Kruz in comparison with reference drugs.